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A simple and low-cost method for fluoride analysis of plant materials using alkali extraction and ion-selective electrode

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Abstract

Backgrounds Existing methods for fluoride (F^-) determination in plant material require expensive equipment and specialized reagents. This study aimed to develop a simple and cost-effective method for fluoride analysis in plant samples.

Results Using an orthogonal assay design with certified reference material, this study optimized a sodium hydroxide extraction method (5 $mol·L^{-1}$) with heating at 120 °C for 0.5 h, followed by the addition of potassium acetate, ionic strength adjustment, and measurement *via* an ion-selective electrode. The method achieved a limit of detection (LOD) and limit of quantification (LOQ) of 1.41 and 4.71 $mg·kg^{-1}$, respectively. Recovery rates ranged from 84.74 to 89.34% in *Arabidopsis thaliana* (intraday relative standard deviation [RSD] ≤ 2.31%, inter-day RSD ≤ 4.17%) and from 83.53 to 91.55% in *Camellia sinensis* (intraday RSD ≤ 3.11%, inter-day RSD ≤ 4.98%). In *A. thaliana* cultivated in NaF-dosed (500 µM) nutrient solution, the fluoride concentration in the shoot was 16.00 $mg·kg^{-1}$; In *C. sinensis* grown under 250 µM NaF treatment, the shoot fluoride concentration was 292.71 $mg·kg^{-1}$. Moreover, the fluoride concentration in Tea products purchased from local supermarkets ranged from 16.28 to 61.78 mg kg^{-1}.

Conclusion This study presents a simple, reliable, and cost-effective method for fluoride analysis in plant materials, which can be further validated through inter-laboratory testing to establish a standardized approach.

Keywords Chemometrics, Orthogonal assay design, Fluoride, Plant research, Ion-selective electrode

Background

Fluorine is the 13th most abundant element on Earth and primarily exists as fluoride (F^-) in salts and organic compounds [1]. Over the past decades, fluoride pollution has intensified due to the increasing anthropogenic activities [2]. Fluoride contamination in groundwater poses a significant public health risk, with the World Health Organization (WHO) estimating that over 200 million people are exposed to levels exceeding the safe limit of 1.5 $mg·L^{-1}$ [3]. High fluoride concentrations (2–45 $mg·L^{-1}$) in regions such as India, Kenya, and Tanzania have been linked to endemic skeletal and dental fluorosis [4, 5]. Additionally, studies indicate that fluoride

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accumulation in crops, particularly Tea (*Camellia sinensis*), can reach toxic levels, posing risks to human health [6]. Tea is a major dietary source of fluoride, with levels ranging from 100 to 800 mg·kg^{-1} in commercial Tea [6–8]. High consumption of brick tea (> 2800 mg·kg^{-1}) has been linked to endemic skeletal fluorosis in the Tibetan regions of China [9]; Surveys in Scandinavia indicated that fluoride exposure among pregnant women gives a high risk of developmental neurotoxicity to their children [10]. Therefore, monitoring fluoride concentrations in plants and food is very important for minimizing the risk of fluoride exposure. However, different methods and techniques largely restrict the agreement of results [11, 12]. Various analytical techniques have been developed to quantify fluoride in environmental and biological samples, differing in sensitivity, accuracy, cost, and complexity. The most widely used methods include ion-selective electrode (ISE) [13], ion chromatography (IC) [14], inductively coupled plasma mass spectrometry (ICP-MS) [15], spectrophotometry [16], and high-resolution continuum source graphite furnace molecular absorption spectrometry (HR-CS-GFMAS). The ISE method uses a F-selective membrane electrode that responds to fluoride in solution, following the Nernst equation. While ISE is a simple and cost-effective approach, proper sample preparation is crucial to mitigate potential interferences (e.g., aluminium and phosphate) [13]. The IC method separates fluoride using a chromatographic column and measures them via conductivity or electrochemical detection. Ion chromatography provides high specificity and sensitivity but requires expensive instrumentation and trained personnel [17]. The ICP-MS method ionizes the analytes of the sample in the plasma and the resulting fluoride is detected based on its mass-to-charge ratio [18]. Spectrophotometry, which relies on colorimetric reactions with fluoride-specific dyes, such as SPADNS (trisodium;4,5-dihydroxy-3-[(4-sulfonatophenyl)diazenyl]naphthalene-2,7-disulfonate), is inexpensive but exhibit limited accuracy in complex matrices such as plant material samples [19]. Moreover, total fluoride detection using HR-CS-GFMAS relies on the formation of diatomic molecules, including CaF_2 and SrF_2 and detection of characteristic molecular absorption [20, 21], but requires costly instrumentation and skilled operation.

The AOAC 975.04 method is a standard approach for determining fluoride content in plant materials *via* acid extraction and ISE measurement [22]. However, acid digestion may not fully release fluoride from strong bonds with calcium and aluminium (elements that are especially high in Tea), leading to underestimation of fluoride content [23]; Additionally, volatile fluorine species can be lost under acidic conditions, and plant constituents such as tannins and polyphenols can interfere with ISE measurements [22, 24]. Although techniques such as microwave digestion [25, 26], oxygen combustion [27], and pyrohydrolysis enhance digestion efficiency [28], they require costly instrumentation, which limit their accessibility in routine analysis. Alkali extraction, particularly with sodium hydroxide (NaOH), offers advantages by improving fluorine liberation, reducing volatilization loss, and minimizing matrix interferences [29, 30]. Aside from the extractant type, the sample weight, extractant concentration, extraction temperature and time, and the percentage of total ionic strength adjusting buffer (TISAB) can significantly influence fluoride measurement [12, 31, 32]. To date, little attempts have been made to optimize these factors through an integrated, multifactor approach. Unlike univariate methods, which vary one factor at a time, multivariate optimization allows for the simultaneous evaluation of multiple variables and their interactions, thereby improving efficiency and robustness of the method development process.

Therefore, this study aims to develop and validate a simple, cost-effective method for fluoride determination in plant materials, focusing on (1) optimizing pretreatment steps, (2) evaluating the range, accuracy, and precision of the method in two representative types of plant samples, and (3) assessing its feasibility for plant research applications. In contrast to methods utilizing complex reagents or expensive equipment, the method proposed here based on alkali extraction andISE measurement offers a low-cost, safer, and more accessible alternative for routine fluoride analysis in plant tissues.

Materials and methods

Apparatus and reagents

Fluoride measurements were performed using a fluoride selective electrode (perfectION™ Combination Fluoride Electrode, Mettler Toledo, Netherlands) coupled to a portable meter (SevenGo pro™, Mettler Toledo, Netherlands). Additional equipment included a balance (Mettler Toledo, Netherlands), a dry block heater (Dry Block Heater 2, IKA, Germany), and plastic tubes for sample preparation. All chemicals used were of analytical grade. Sodium hydroxide (≥ 99.5%), potassium acetate (≥ 99.0%), sulfuric acid (95–98%), nitric acid (70%), chloric acid (37%), and phosphoric acid (85%) were purchased from Merck. Sodium hydroxide (4 mol·L^{-1}, pH 5.0) and TISAB I was prepared according to the manufacturer guidelines. Standard solution of NaF (201154, Sigma-Aldrich) was prepared by dissolving in ultrapure water to make up the stock solution (1000 mg·L^{-1}) and stored at 4 °C. The certified reference material (CRM) of Tea (GBW10016) was obtained from the National Institute of Metrology, China.

Plant growth and fluoride dose treatment

Seed of *Arabidopsis thaliana* (ecotype Col-0) was germinated and seedlings were grown in half strength Hoagland Gelzan gel for 10 d and then transferred to polyethylene pots (three plants per pot) filled with 500 mL of half strength Hoagland solution (at pH 5.5). After growing in the aerated solution for 7 d, different dose levels of fluoride were added into the solution and the plants were allowed to take up fluoride for another 7 d. Tea (*Camellia sinensis* var. *sinensis*) seeds (OnsZaden, Wageningen, Netherlands) were scarified with sandpaper and soaked in tap water for three days. The seeds were then buried in a moist mix of 50/50 perlite/vermiculite for five to seven weeks (emerged seedlings had 3–4 leaves) and then transferred to polyethylene pots (three plants per pot) filled with 4 L half strength Hoagland solution (at pH 5.5). After growing in the aerated solution for 2 months, different dose levels of fluoride were added to the solution and the plants were allowed to take up fluoride for another 2 months. Fluoride concentrations were selected based on previous studies on fluoride accumulation in plants [33, 34].

The *A. thaliana* and *C. sinensis* seedlings were grown under a regime of 12/12 h photoperiod set at a light intensity of 150 μmol m^{-2} s^{-1} and a relative humidity of 70% at 20 ± 2 °C. At the end of the growth phase, plant samples were harvested at the previously specified time using scissors and then dried at 65 °C for three days. The dried plant material was powdered with a batch mill (Tube Mill 100 control, IKA, Germany) at 20,000 rpm for 1 min. Commercial Tea samples, including green and black teas, were purchased from local supermarkets and represented seven different brands: Albert Heijn (bio rooibos puur, engelse melange, bio groene thee puur, groene thee puur), Clipper (pure organic green tea), Jumbo (engelse melange, groene thee puur), Lipton (green tea, yellow label), Lidl (English breakfast), Pickwick (green tea, original earl grey, original ceylon), and Twinings (original english breakfast).

Orthogonal assay design

The orthogonal assay design followed an $L_{18}(3^6)$ matrix, evaluating three levels of sample weight (20 mg, 100 mg, 200 mg), extractant type (NaOH, H_2SO_4, H_3PO_4), extractant concentration (0.5 mol·L^{-1}, 1 mol·L^{-1}, 5 mol·L^{-1}), extraction time (0.5 h, 1 h, 2 h), extraction temperature (70℃, 100℃, 120℃), and the ratio of TISAB I (1:2, 1:1, 2:1). The relative recovery of the CRM was used to assess optimization efficiency.

General analytical procedures

First, 20 mg of sample material was weighed and transferred into a 10-mL polypropylene centrifuge tube. Then 5 mL of 1 mol·L^{-1} NaOH was added and the mixture shaken by hand for 10 s and placed in a preheated hot block at 120 °C for 1 h. Following on, the tubes were taken out and cooled down to room temperature and centrifuged at 4,000 rpm for 10 min. Then, 2 mL of the supernatant was transferred to a new 10 mL tube, and 3 mL of potassium acetate solution was added to adjust the pH to 5.0–5.5. Potassium acetate was chosen over other strong acids to avoid the formation of HF and potential fluoride loss. In addition, the use of acetate buffer maintains ionic strength while providing a mild pH adjustment compatible with fluoride ISE measurement. Finally, 5 mL TISAB I solution was added in the solution to adjust the ionic strength.

Establishment of calibration curves

The measured potential from the ionic-selective electrode corresponding to the level of F^- in solution is described by the Nernst equation: $E = S \log C + B$. Where C is the [F^-] in mg·L^{-1}, yielding a millivolt potential of E, S is the slope, and B is the reference potential. Calibration curves were prepared by spiking fluoride (0.03, 0.10, 0.30, 1.00, and 3.00 mg·L^{-1}) into a blank solution (1 mol·L^{-1} NaOH) before extraction and tested in between plant material samples. Finally, the corresponding values of the potential was regressed.

Determination of limits of detection, quantification, and range

The limit of detection (LOD) and limit of quantification (LOQ) values were calculated based on the ICH Q2 (R2) guideline [35], and the formulae were as follows: $LOD = \frac{3.3\times\sigma}{S}$ and $LOQ = \frac{10\times\sigma}{S}$. σ is the standard deviation of the blank and S was the slope of the calibration curve. The calibration curve was constructed using F standard solutions ranging from 0.03 to 3.00 mg·L^{-1} to cover both low and high expected sample concentrations.

Determination of recovery, relative recovery, accuracy, and precision

The recovery (%) was calculated by ($C_{Observed}$/C_{CRM})×100%. The relative recovery was calculated: $\frac{1}{abs\left(\frac{Actual\ conc - CRM\ conc}{CRM\ conc}\right)}$.The accuracy tests were carried out by spiking 25, 50, 100 mg·kg^{-1} standards to a sample blank before extraction. Intraday tests (precision) were assessed by analysing the same sample six times within a single day, while interday tests were evaluated by analysing the same sample once daily over three consecutive days. The precision was showed in relative standard deviations (RSD, %). The test was conducted on a sample blank that was spiked to achieve three different fluoride concentrations with six replicates measured on three separate days. The recovery was calculated based on the exact concentration divided by the theoretical concentration.

Statistical analyses

Statistical significance was assessed using Student's *t*-tests for pairwise comparisons and one-way ANOVA with Duncan's Multiple Range Test for group comparisons (SPSS Version 24, IBM, USA). Results were considered statistically significant at $P < 0.05$.

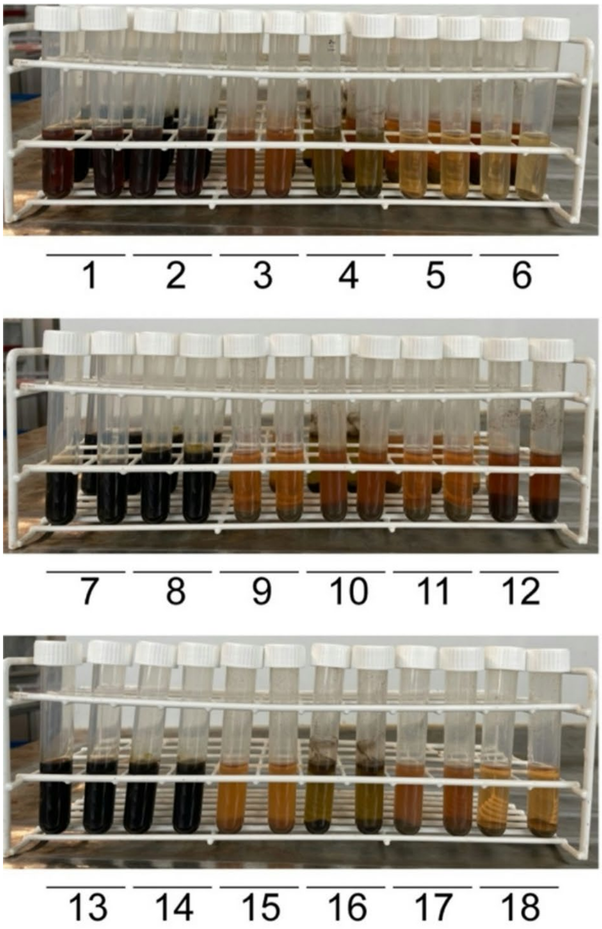
Results and discussion

Orthogonal assay design

In this study, our aim was to establish a method that is not only effective but also safe, low-cost, and accessible, particularly for labs in plant research settings. Acidic reagents (H_2SO_4 and H_3PO_4) were included in the preliminary screening phase to serve as a comparison. However, acid-based F extraction methods have known limitations, such as potential fluoride loss due to HF volatilization or precipitation with metal ions [23]. Alkaline agents like ammonia and tetramethylammonium hydroxide were not considered, owing to their volatility, toxicity, and limited accessibility. Sodium hydroxide (NaOH) was ultimately selected for its strong extraction efficiency, chemical stability, and broad accessibility. An orthogonal assay design was employed to optimize key parameters affecting fluoride extraction. The tea CRM (57 ± 15 mg·kg^{-1}) was used to calculate the recoveries of these combination, which has been reported as a reliable reference [26, 36]. Before adding potassium acetate, an obvious difference in appearance was observed among the combinations of extractants tested. The colour and clarity of supernatant was found to be significantly influenced by the extractant type; from black-turbid (NaOH extractant) to yellow-clear (H_3PO_4 extractant), whereas the H_2SO_4 extractant made samples difficult to separate from the supernatant with centrifugation (Fig. 1).

Optimization of analytical procedures

The fluoride concentration has large differences ranging from 0.9 to 136.0 mg·kg^{-1} (Table 1). Among them, the recoveries of seven combinations (2, 4, 6, 7, 8, 16, 18) was within 80–120%. We next use the relative recovery to quantify the accuracy, on the understanding that



1: 20 mg, NaOH, 0.5 M, 0.5h, 70°C, 0.5:1
2: 20 mg, NaOH, 1 M, 1h, 120°C, 2:1
3: 20 mg, H_2SO_4, 0.5 M, 2.5h, 120°C, 1:1
4: 20 mg, H_2SO_4, 5 M, 0.5h, 100°C, 2:1
5: 20 mg, H_3PO_4, 1 M, 2.5h, 100°C, 0.5:1
6: 20 mg, H_3PO_4, 5 M, 1h, 70°C, 1:1
7: 100 mg, NaOH, 0.5 M, 2.5h, 100°C, 2:1
8: 100 mg, NaOH, 5 M, 0.5h, 120°C, 1:1
9: 100 mg, H_2SO_4, 1 M, 1h, 100°C, 1:1
10: 100 mg, H_2SO_4, 5 M, 2.5h, 70°C, 0.5:1
11: 100 mg, H_3PO_4, 0.5 M, 1h, 120°C, 0.5:1
12: 100 mg, H_3PO_4, 1 M, 0.5h, 70°C, 2:1
13: 200 mg, NaOH, 1 M, 2.5h, 70°C, 1:1
14: 200 mg, NaOH, 5 M, 1h, 100°C, 0.5:1
15: 200 mg, H_2SO_4, 0.5 M, 1h, 70°C, 2:1
16: 200 mg, H_2SO_4, 1 M, 0.5h, 120°C, 0.5:1
17: 200 mg, H_3PO_4, 0.5 M, 0.5h, 100°C, 1:1
18: 200 mg, H_3PO_4, 5 M, 2.5h, 120°C, 2:1

Fig. 1 Visual appearance of sample mixtures using various extractant solutions in 18 combinations and the details of each combination

Table 1 The $L_{18}(3^6)$ matrix associated with the concentrations and recoveries of CRM

Combination	Factor						Concentrations	Recovery (%)
	A	B	C	D	E	F	(mg·kg^{-1})	
1	1	1	1	1	1	1	0.9	1.53
2	1	1	2	2	3	3	54.4	95.41
3	1	2	1	3	3	2	132.2	231.88
4	1	2	3	1	2	3	53.5	93.89
5	1	3	2	3	2	1	28.5	49.99
6	1	3	3	2	1	2	47.5	83.26
7	2	1	1	3	2	3	47.7	83.68
8	2	1	3	1	3	2	53.4	93.62
9	2	2	2	2	2	2	115.9	203.39
10	2	2	3	3	1	1	27.8	48.71
11	2	3	1	2	3	1	42.9	75.26
12	2	3	2	1	1	3	96.1	168.67
13	3	1	2	3	1	2	38.3	67.19
14	3	1	3	2	2	1	18.7	32.79
15	3	2	1	2	1	3	136.0	238.62
16	3	2	2	1	3	1	48.4	84.99
17	3	3	1	1	2	2	70.0	122.87
18	3	3	3	3	3	3	64.8	113.76

Notes: Factor from A to F was (A) sample weight, (B) extractant type, (C) extractant concentration, (D) extraction time, (E) extraction temperature, and (F) the percentage of TISAB, respectively. Details of each combination was described in the Fig. 1

Table 2 ANOVA for output responses in the $L_{18}(3^6)$ matrix

Source	SS	df	MS	F-value	Significance
A	52.696	2	26.348	0.973	0.44
B	58.585	2	29.293	1.081	0.407
C	84.613	2	42.306	1.562	0.297
D	49.824	2	24.912	0.92	0.457
E	148.721	2	74.361	2.745	0.157
F	113.789	2	56.894	2.1	0.218
Error	135.452	5	27.09		
Total	1217.95	18			

Notes: Factor from A to F was (A) sample weight, (B) extractant type, (C) extractant concentration, (D) extraction time, (E) extraction temperature, and (F) the percentage of TISAB, respectively. SS, sum of square; df, degrees of freedom; and MS, mean square.

the higher the relative extraction efficiency, the higher the accuracy will be. We evaluated the significance of the effects of different parameters on the relative recovery using ANOVA. Table 2 shows that no statistically differences were observed among the six parameters ($P>0.05$), suggesting that all six parameters have a substantial effect on fluoride measurement. Moreover, no significant interaction was observed, suggesting that each parameter independently contributes to fluoride measurement. Then, range analysis is applied to clarify the significance levels of six influencing factors on the relative recovery [37]. Results showed that the extraction temperature has the largest influence on the accuracy among the six tested parameters, followed by the percentage of TISAB added, the extractant concentration, the extraction temperature, the sample weight, and the extractant type (Table 3). Higher extraction temperatures are believed to promote the breakdown of plant tissue matrices and enhance fluoride release, which is why numerous studies have employed a muffle furnace or microwave-assisted digestion systems for efficient sample preparation. Consistent with previous findings,

Table 3 Range analysis for output responses in the $L_{18}(3^6)$ matrix

Level	A	B	C	D	E	F
K1	47.883	49.122	17.038	45.531	14.163	17.156
K2	30.210	27.417	35.908	34.968	31.315	30.785
K3	23.559	25.113	48.706	21.152	56.173	53.712
k1	15.961	16.374	5.679	15.177	4.721	5.719
k2	10.070	9.139	11.969	11.656	10.438	10.262
k3	7.853	8.371	16.235	7.051	18.724	17.904
R	8.108	8.003	10.556	8.126	14.003	12.185

Notes: Factor from A to F was (A) sample weight, (B) extractant type, (C) extractant concentration, (D) extraction time, (E) extraction temperature, and (F) the percentage of TISAB, respectively. K indicates the sum of the data for each factor at each level; k indicates the average of the data for each factor at each level; R indicates the difference between the maximum and the minimum values of k.

a reduced volume of TISAB was found suitable for fluoride analysis [26], suggesting that interfering cations are effectively complexed. However, given that the fluoride concentration we tested is only 57 mg·kg^{-1}, further investigation is needed to determine whether a lower volume of TISAB would suffice for samples with higher fluoride concentration. The acid extraction method proposed by the AOAC is both simple and cost-effective, utilizing only diluted HNO_3 (0.05 M) as the extractant, followed by direct measurement of fluoride after adjusting pH [22]. The limitations of this method include its inability to release fluoride complexes in plant samples through acid digestion [38] and potential for incomplete lyophilization of the sample without heating. Our method for determining fluoride is analogous to the AOAC method, and we also observed that the relative recovery achieved through alkali extraction significantly surpasses that of acid extraction, confirming that acid digestion is not suitable for fluoride detection in plant samples.

Based on the range analysis, the theoretically optimal conditions were determined by selecting the highest-level values of each parameter. This combination consisted of 20 mg of sample extracted with 5 mol·L^{-1} NaOH, heated at 120 °C for 0.5 h, followed by the addition of TISAB I in a 1:1 ratio (corresponding to A1B1C3D1E3F3). The recovery of the theoretically optimized combination was 103% (58.5 ± 2.08 mg·kg^{-1}), suggesting that the orthogonal array design has achieved optimization and therefore the next experiments were based on this combination.

Linearity and range

By spiking a range of fluoride concentrations into the solutions, we could calibrate our method ($Y=-59.943X+96.291$, $R^2=0.9994$). Linearity was achieved in the range of 0.03–3.00 mg·L^{-1}. The LOD and LOQ were 1.4 and 4.7 mg·kg^{-1}, respectively. However, according to the probe manufacturer's manual, the lowest level of detection is 0.02 mg·L^{-1}, one should be careful when measuring below 0.02 mg/L. The LOD and LOQ obtained are within the interval of reported data in previous studies using acid digestion and alkali fusion for fluoride determination [26, 39]. Additionally, the fluoride concentrations in *A. thaliana* and *C. sinensis* are in the same order of magnitude compared to other published studies [40, 41]. While matrix-matched calibration curves can reduce matrix effects, their necessity depends on the sample composition and feasibility of matrix preparation. Tea plants are known to accumulate high concentrations of fluoride in a complex metal (Al, Ca, Mn) ion matrix [42]. Preparing a reliable and representative matrix for all samples proved impractical and posed risks of inconsistency and background fluoride contamination. Furthermore, we observed that even Tea seedlings grown under fluoride-free conditions had leaf fluoride concentrations exceeding 15 mg·kg^{-1} (which can be explained due to the seed fluoride store). Reducing this to a suitable blank level for calibration would require significant dilution, thereby compromising matrix reliability. Therefore, matrix-matched calibration was not considered appropriate for this study.

Table 4 Accuracy and precision of the analysis of fluoride concentrations in *A. thaliana* and *C. sinensis* plant materials

Matrix	Spiked level (mg·kg^{-1})	Recovery % (Mean ± SD)	Within-day RSD (%, *n* = 9)	Between-day RSD (%, *n* = 9)
A. thaliana	10.0	85.71 ± 0.91	1.06	4.14
	25.0	89.34 ± 1.32	1.46	3.24
	50.0	84.74 ± 1.96	2.31	4.17
C. sinensis	10.0	83.53 ± 2.31	2.96	4.54
	25.0	88.41 ± 2.22	2.48	4.24
	50.0	91.55 ± 2.96	3.11	4.98

Accuracy and precision

Fluoride determination in plant materials is susceptible to matrix interferences due to the presence of organic acids, polyphenols, and metal ions. They have been reported to form complexes with fluoride, potentially reducing their free concentration in solution [8, 31, 38]. Following the ICH Q2(R2) guideline, method accuracy was assessed through spike recovery tests, and precision was evaluated based on repeatability. In *A. thaliana*, the recovery ranged from 84.74 to 89.34%, with no significant differences observed among the different spiking levels. The within-day relative standard deviation (RSD) was ≤ 2.31%, and the between-day RSD was ≤ 4.17%. In *C. sinensis*, the recovery ranged from 83.53 to 91.55%. The recovery of the higher spike level was significantly greater than that of the lower level ($P < 0.05$, Tukey's test). The within-day and between-day RSDs were ≤ 3.11% and ≤ 4.98%, respectively (Table 4). These results demonstrate that the method has a high level of accuracy and precision. Alkali treatment with NaOH effectively releases fluoride from bound complexes, while adding potassium acetate ensures optimal fluoride solubility. Additionally, the use of TISAB helps complex interfering metal ions, stabilizing fluoride activity in solution.

To comprehensively evaluate this method, the analytical parameters including LOD, linear range, accuracy, and precision, costs, and simplicity of proposed method were compared with previous studies (Table 5). Compared to existing methods such as AOAC 975.04, ICP-MS, and ion chromatography, this method provides a cost-effective, efficient, and highly reproducible alternative with minimal equipment requirements. Unlike acid digestion-based methods, which risk fluoride volatilization and underestimation, this approach using alkali extraction enhances fluoride recovery while minimizing

Table 5 Fluoride analytical methods comparison for analysis of plant materials

Method (ref)	LOD (mg·kg^{-1})	Linear Range (mg·L^{-1})	Accuracy (%)	Precision (RSD, %)	Estimated cost ($/sample)	Advantages	Disadvantages
This study	1.41	0.03–3.0	84–92	≤ 5	$1–2	Simple, low-cost, accurate, minimal interference	Requires optimization for different matrices
AOAC 975.04 [22]	2	0.1–10	80–95	≤ 10	$2–5	Widely accepted, simple procedure	Incomplete F release, potential loss
IC [14]	0.1	0.05–5.0	85–98	≤ 3	$10–20	High specificity and sensitivity	Expensive equipment, trained personnel required
ICP-MS [15]	0.01	0.001–10	90–99	≤ 2	$30–50	Extremely sensitive, detects trace levels	Very expensive, requires skilled personnel
Spectrophotometry [16]	0.5	0.2–2.0	75–90	≤ 8	$0.5–2	Low-cost, simple, suitable for liquids	Low accuracy in complex matrices
Microwave Digestion + ISE [26]	0.8	0.05–5.0	85–97	≤ 4	$5–15	Efficient extraction, high accuracy	Expensive setup, longer preparation time

Table 6 Concentrations of F$^-$ in Tea products from supermarkets in the Netherlands

Sample ID	Tea type	Fluoride concentration mg·kg^{-1} (mean ± SD, *n* = 3)
1	Black tea	16.3 ± 3.72
2	Black tea	47.4 ± 3.90
3	Green tea	33.1 ± 3.42
4	Green tea	40.3 ± 2.70
5	Green tea	61.8 ± 3.73
6	Black tea	37.8 ± 6.31
7	Green tea	24.2 ± 1.93
8	Green tea	79.3 ± 6.23
9	Black tea	37.6 ± 2.17
10	Black tea	46.6 ± 3.01
11	Green tea	30.5 ± 1.71
12	Black tea	23.7 ± 0.18
13	Black tea	20.4 ± 0.95
14	Black tea	33.8 ± 2.16

Note: these Tea products were randomly selected from three different supermarkets in the Netherlands and all of them were pure Tea (without herbs, fruits, or spices).

interference from plant constituents. Additionally, the method is user-friendly, making it accessible for routine laboratory analysis without the need for highly specialized instrumentation.

Determination of fluoride in plant materials

The fluoride concentrations of *A. thaliana* and *C. sinensis* cultivated in fluoride-dosed nutrient solution was determined, and this shows that the fluoride concentration in the shoot of *A. thaliana* increased with increasing fluoride dose levels from 0.6 to 16.0 mg·kg^{-1}, respectively (Fig. 2a). The measured values are consistent with previously reported fluoride concentrations in *A. thaliana*, which range from 0 to 20 mg·kg^{-1} [40]. In *C. sinensis*, the fluoride concentration in the shoot was 292.7 mg·kg^{-1} following the fluoride-dosing treatment (Fig. 2b), which is lower than a previously reported value of approximately 1000 mg·kg^{-1} under hydroponics conditions [42]. This discrepancy may be attributed to differences in cultivar and plant age. Moreover, differences in fluoride accumulation between *A. thaliana* and *C. sinensis* can be attributed to their distinct root uptake mechanisms and fluoride-binding capacities. Tea plants accumulate high fluoride levels due to their high Al contents, which facilitates fluoride complexation [42]. In contrast, *A. thaliana* has limited fluoride transport and shows a dose-dependent accumulation pattern [34, 40].

Additionally, we tested 14 different Tea products from local supermarkets (Table 6). The results show fluoride concentrations ranging from 16.3 to 61.8 mg·kg^{-1}. which are consistent with previous findings, a study reporting a mean fluoride content of 85.16 mg·kg^{-1} in 558 commercial Tea samples [43]. While the WHO sets a fluoride limit of 1.5 mg·L^{-1} in drinking water, actual fluoride concentrations in tea infusions in Europe have been reported to range from 0.3 to 8.9 mg·L^{-1} [44], underscoring the importance of monitoring and controlling fluoride content in Tea products.

Conclusions

This study successfully developed a simple, low-cost, and reliable method for fluoride determination in plant materials using alkali extraction and ISE measurement. The method was successfully applied to diverse plant types, including *A. thaliana*, *C. sinensis*, and commercial Tea samples. Furthermore, the use of orthogonal design enabled the systematic identification of factor interactions, contributing to a robust and reproducible protocol for fluoride quantification in plant tissues. Compared to previous methods, our study enhances fluoride liberation, minimizes interference, and reduces equipment costs. While inter-laboratory validation is necessary for broader adoption, this method holds significant promise for fluoride monitoring in plant-based research and food safety applications.

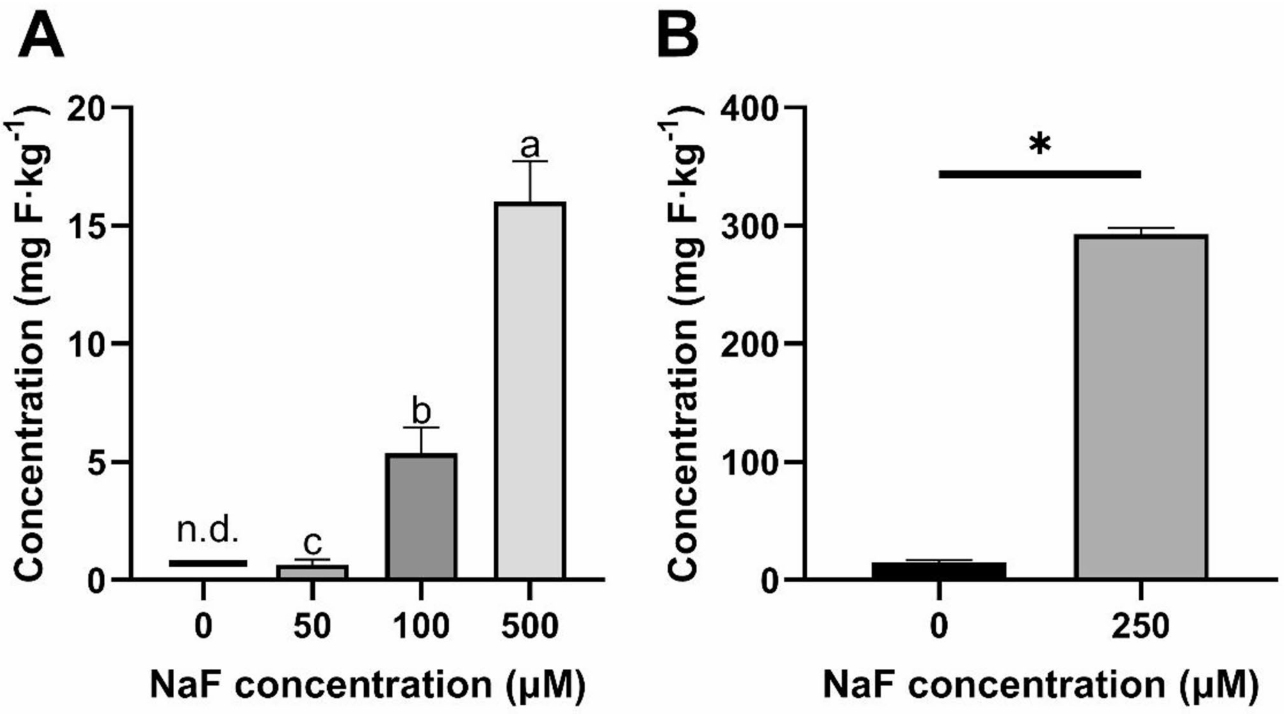


Fig. 2 Concentrations of fluoride in *A. thaliana* and *C. sinensis* plants dosed with F^- in hydroponic solution. (A) Fluoride concentrations in *A. thaliana*; (B) Fluoride concentrations in *C. sinensis*. N.D. not detected, indicates data were below the limit of quantification. Different letters and * indicate significant difference ($P<0.05$) among treatments

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Author contributions
CZ: conceptualization, investigation, data analysis and interpretation, writing original draft. MGMA: writing and review. AvdE; Conceptualization, investigation and editing.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics and consent to participate declarations
Not applicable.

Consent to publish
Not applicable.

Competing interests
The authors declare no competing interests.

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